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Effects of Interstitial Heating on the RIF-1 Tumor Using an Nd:YAG Laser With Multiple Fibers

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Background and Objective: **Hyperthermia was induced in tumor-bearing C_3H mice using a Nd:YAG laser emitting near-infrared radiation at 1,064 nm. The efficacy of multiple implanted fiberoptics in the control of the RIF-1 tumor was investigated.**
Study Design/Materials and Methods: **RIF-1 tumors in the right hind leg were heated interstitially at 42, 44, or 46°C for 30 or 60 minutes. Two, three, or four 400-μm quartz fibers terminating in a 1.0-cm cylindrical diffusor were inserted into each tumor, as were five microthermocouples to monitor temperature during treatment. Laser Doppler Flow (LDF) was also recorded pre- and post-treatment to determine changes in red blood cell flux in overlying skin (42, 44, or 46°C) and the center of the tumor (46°C).**
Results: **These experiments indicated that interstitial heating at 42, 44, and 46°C resulted in tumor growth delay, although long-term control of tumors was not achieved. Treatment using four fibers resulted in the greatest tumor growth delay at 42 and 44°C, increasing tumor doubling time by 50% or greater compared to control tumors; tumor growth delay following 46°C treatments was seven times greater than that in control tumors. Significant changes (decreases) in LDF ($P < .05$) were seen in four treatment groups, using two fibers at 42°C for 30 minutes, four fibers at 44 and 46°C for 60 minutes on the overlying skin, and 46°C for 60 minutes in the center of the tumor.**
Conclusions: **Initial data indicate that interstitial heating with multiple fibers increases tumor growth delay compared to previous single fiber treatments, with tumor growth delay increasing with increasing treatment temperature; however, long-term tumor control was not achieved under the conditions investigated. Follow-up studies will explore the use of higher temperatures and/or longer treatment times in order to optimize tumor response.**

Key words: fibrosarcoma, infrared heating, laser Doppler flow, necrosis, tumor growth delay

INTRODUCTION

The treatment of tumors via increased temperature (hyperthermia) has been the subject of renewed interest over the past decade, with a number of recent studies supporting its value as a therapeutic agent [1–6]. Hyperthermia treatment may cause preferential damage to cancer cells; temperatures ranging from 42 to 45°C have the ability to cause irreversible damage in malignant cells while causing little or no damage to surrounding normal tissues. Indirect tumor cell death can result from decreases in oxygen tension and cellular pH [7,8], while lethal events in the tumor cell itself, such as inhibition of DNA, RNA

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and protein synthesis, changes in tumor cell metabolism, and cytoplasmic destruction [8] can lead to direct tumor cell death.

The ability of elevated temperatures to cause such damage within a tumor has often been targeted toward the tumor vasculature [2,5,9,10]. In addition to causing damage to cellular structures, hyperthermia has also been shown to decrease tumor blood flow during or post-heating [11]. Decreases in tumor blood flow following hyperthermia treatment may provide an indication of the initial vascular damage induced in the tumor as well as the long-term prognosis for tumor control. The vasculature system of a tumor appears poorly formed in comparison to that of normal tissues, possessing several distinguishing characteristics that make it more sensitive to hyperthermia [2,12]. As tumor size increases, the vasculature system often begins to deteriorate owing to lack of adequate space, thus decreasing blood flow within the tumor. In healthy tissues, heat is lost by convection (blood flow); in such instances, normal vasculature is able to prevent excessive heating of the tissue [13]. Since tumors do not have sufficient blood circulation, the dissipation of heat within the tumor cannot occur; the hypoxic and necrotic regions have the ability to retain more heat and, thus, reach higher temperatures than in normal tissue [7]. The resulting increase in temperature may lead to the preferential destruction of surrounding tumor tissue.

Several groups have utilized near-infrared laser radiation for the induction of hyperthermia. The most adequate laser at present to produce such radiation appears to be the Nd:YAG laser, which emits energy at a wavelength of 1,064 nm. This wavelength produces a typical 1/e optical penetration depth of $\geq$5 mm, depending on the tissue and method of delivery [14]. Interstitial delivery of this energy can provide relatively uniform heating of the tumor mass. If the tumor mass is not adequately heated, some tumor cells retain the ability to divide, thus leading to tumor regrowth.

This present study was performed as a continuation of a previous study [4], to further evaluate RIF-1 tumor response following the use of multiple fiber hyperthermia as opposed to single fiber treatment. Our objectives were to determine the best possible method of delivery of heat (i.e., the number and arrangement of fibers), to assess the relevance of changes in laser Doppler flow before and after heating as an indicator of treatment outcome, and to determine the future prospect of using such methods in the treatment of human tumors.

MATERIALS AND METHODS

Animal Tumor Model

The animal tumor model used in this study was the RIF-1, a heat-resistant, weakly immunogenic, radiation-induced fibrosarcoma. Since this tumor system is minimally immunogenic and does not produce early spontaneous metastases, it is considered an ideal tumor system for comparing tumor response endpoints [15]. This model was obtained from Roswell Park Cancer Institute (Buffalo, NY) and maintained in accordance with previously established protocols [15]. Male C_3H mice weighing 18–20 g (Charles River Laboratories, Wilmington, MA) were inoculated subcutaneously into the right hind leg (previously shaved) with $1–2 \times 10^6$ cells. Animals were housed under standard American Association for Accreditation of Laboratory Animal Care (AAALAC)-approved conditions, fed mouse chow and water ad libitum, and maintained under a constant 12-hour light/dark cycle. Once the ellipsoid-shaped tumors reached a size of 10×10 mm (estimated volume 400 mm^3, as measured with vernier calipers) approximately 12–14 days post-inoculation, heat treatment was performed. Prior to treatment, tumor-bearing mice were anesthetized with 0.035 cc I.M. of ketamine HCl (100 mg/ml) and placed in a plastic restrainer that exposed the tumor-bearing limb.

Laser System and Temperature Measurements

A Model 4000 Cardiolase™ microprocessor-controlled Nd:YAG laser (Trimedyne, Tustin, CA) emitting near-infrared laser energy at 1,064 nm was the source of hyperthermia used in this study. This is a continuous-wave, air-cooled laser with a power output of 1 to 55 Watts; it can be adjusted in 1-Watt increments. The desired power output is set prior to operation (for these experiments, a power of 8.0 Watts was used). Interstitial delivery of hyperthermia occurred via two, three, or four flexible 400-μm quartz fibers terminating in 1.0-cm cylindrical diffusors (PDT Systems, Inc., Goleta, CA). The diffusors were connected to the laser by an independent beam splitter device (Line Lite Laser Corp., Mountain View, CA). The primary laser beam enters the beam splitter, where it is subsequently split into as many as four independent beams. Each output port of the beam splitter is coupled to a fiberoptic that delivers

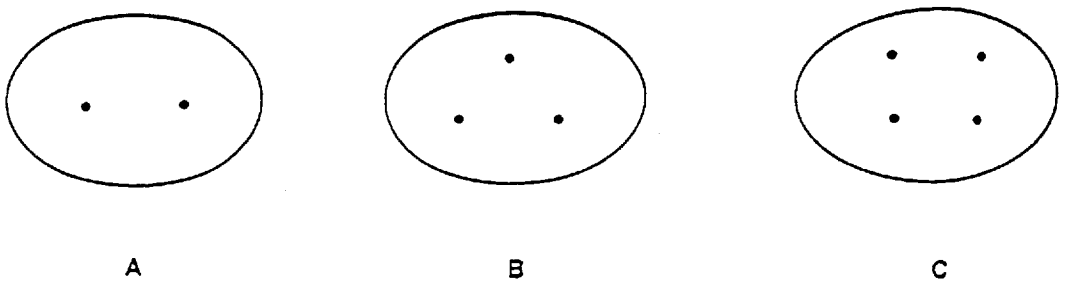

Fig. 1. Orientation of two (A), three (B), or four (C) fibers implanted for heat treatment in RIF-1 tumors.

heat to the tumor mass. The cylindrical diffusors were inserted into the tumor in one of three configurations (Fig. 1).

Temperature measurements were made in the tumor using five 0.005-inch Copper/constantan microthermocouples (Omega, Stamford, CT) in order to record accurate temperatures representative of the entire tumor. Four of the thermocouples operated in conjunction with a microprocessor program that controlled laser output during treatment. The desired treatment temperature (either 42, 44, or 46°C) was set prior to treatment; the thermocouples were placed within 1.0 mm of each fiber. When each treatment started, these thermocouples monitored the temperatures generated in the proximity of each fiber in use. The subsequent temperature readings were conveyed to the microprocessor program which then automatically adjusted the laser power output to achieve the desired temperature, usually resulting in temperatures within 0.5°C of the treatment parameter. A fifth microthermocouple, attached to a digital temperature readout (Physitemp, Clifton, NJ) was placed in the center of the tumor, to provide additional temperature readings during heating. The small diameters of each fiber and microthermocouples allowed their use in various configurations without causing unnecessary trauma in the tumor mass.

Laser Doppler Flow Measurement

Red blood cell flux (laser Doppler flow or LDF) was measured in overlying skin or in the center of each tumor using a Laserflo™ blood perfusion monitor (Vasamedics, Inc., Model BPM 403A, St. Paul, MN). This device emits 1.5 mW of near-infrared radiation at 780 ± 20 nm. A single-needle probe of 0.8 mm tip diameter was secured by a micromanipulator on the surface (skin) or in the center of the tumor 30 minutes prior to treatment. Readings of relative LDF were taken every 3 minutes before treatment and every 3 minutes for 30 or 60 minutes post-treatment. LDF measurements could not be taken during actual heat treatment because of an interaction between the Nd:YAG laser wavelength (1,064 nm) and the LDF wavelength (780 nm). For treatment groups in which LDF measurements were taken on the surface of the skin, the LDF probe remained on the surface of the skin during the entire heat treatment to prevent any unnecessary trauma to the tumor. For the treatment group in which LDF measurements were taken in the center of the tumor it was necessary to remove the probe from the center of the tumor during treatment because of wavelength interactions.

Treatment Groups

Each treatment group consisted of ten tumor-bearing mice. The tumor volume of all RIF-1 tumors prior to treatment was 400 mm^3. Tumors were heated for 30 or 60 minutes at 42, 44, or 46°C. Two, three, or four quartz fibers were inserted into the tumors treated at 42 or 44°C; 46°C treatments were performed exclusively with four fibers. Five microthermocouples were inserted into each tumor for all treatments.

Tumor Response and Data Analysis

Tumor response was assessed by necrosis present on the surface of the tumor and by subsequent growth delay of the tumor following heat treatment. Necrotic measurements were taken on the surface of the tumor within 48 hours post-treatment. The amount of necrosis present at 48 hours provides a gross indication of the initial damage to the tumor. The surface areas of the tumor and the section of necrosis were determined using the equation $4\pi r^2$, where r was the radius of the tumor; the surface area of the scab was divided by the surface area of the tumor to determine percent necrosis.

The efficacy of heat treatment was determined by comparing treated RIF-1 tumor growth

to that of untreated RIF-1 tumors. Tumor volume was calculated using the formula $V = 0.4 \times ab^2$, where a and b were tumor diameters (longer and shorter, respectively) as measured with vernier calipers. The mean time for untreated RIF-1 tumors to grow from 400 to 800 mm^3 was 3.4 days (data not shown). Tumor growth delay (TGD) was determined by comparing the growth rate of the treated RIF-1 tumors to the untreated control tumors. Following treatment, the tumors were measured three times per week until the tumors had doubled in size (from 400 to 800 mm^3) or remained non-palpable (flat) for 60 days. Once this endpoint was reached, the mice were sacrificed by cervical dislocation after mild sedation, in accordance with recommendations from the American Veterinary Medical Association. Partially responding tumors were then surgically removed with skin intact and fixed in 10% formalin. This facilitated the determination of necrotic areas within the tumor for each treatment condition employed.

Tumor temperatures (°C) and total doses (J/cm) were recorded over the entire treatment period; mean tumor temperature ± SD and total dose ± SD were calculated for each tumor. Tumor growth delay was determined using linear regression; Pearson's correlation coefficient was calculated to determine the significance of growth delay. Comparisons were made between laser Doppler flow measurements taken 30 minutes pre-treatment and 30 or 60 minutes post-treatment; significance was determined statistically using a two-tailed *t*-test.

RESULTS

Table 1 presents results following heating of RIF-1 tumors at 42, 44, and 46°C. During heat treatment, mean tumor temperature was maintained within 0.3 to 0.6°C of the desired treatment temperature. Temperature profiles included in this study indicate the ability of the microprocessor program to monitor and control tumor temperatures at multiple locations (Figs. 2–5). Little variation was seen among the temperatures at each thermocouple; the mean time for tumors to reach the desired temperature of 42, 44, or 46°C was 6 minutes. Total doses delivered for treatments ranged from 799 to 1,942 J/cm.

It was observed from TGD (Table 1) and laser Doppler flow data (Table 2) that as treatment temperature increased and more fibers were used to deliver heat, more favorable results were seen.

TABLE 1. Treatment Parameters and Response of RIF-1 Tumors to Nd:YAG Laser-Induced Heating*

Temp./Time/No. fibers	Mean temp (°C) ± SD[a]	Total dose (J/cm) ± SD[b]	TGD (days)[c]
42°C/30'/2	42.5 ± 0.24	996 ± 249	7.3 ± 1.5
42°C/30'/3	42.5 ± 0.21	841 ± 254	6.9 ± 1.5
42°C/30'/4	42.6 ± 0.17	799 ± 308	7.6 ± 2.0
42°C/60'/4	42.4 ± 0.11	1,129 ± 204	8.9 ± 2.4
44°C/30'/2	44.4 ± 0.14	1,167 ± 396	8.6 ± 2.6
44°C/30'/3	44.5 ± 0.24	1,090 ± 253	8.3 ± 2.7
44°C/30'/4	44.5 ± 0.28	1,005 ± 149	11.4 ± 3.5
44°C/60'/4	44.3 ± 0.15	1,489 ± 255	10.6 ± 1.8
46°C/60'/4[d]	46.2 ± 0.10	1,634 ± 302	20.8 ± 1.9
46°C/60'/4[e]	46.4 ± 0.20	1,942 ± 300	22.5 ± 2.0

*Ten tumors treated per group.
[a]Mean temperatures (±SD) for four microthermocouples placed 1.0 mm away from each diffusor and one microthermocouple placed in the center of each tumor.
[b]Total dose (±SD) delivered during the 30- or 60-minute treatment.
[c]Calculated as the time in days for treated RIF-1 tumors to double in volume (from 400 to 800 mm^3) compared to untreated tumors.
[d]Fifteen tumors treated.
[e]Fifteen tumors treated; LDF measurements taken in the center of the tumor.

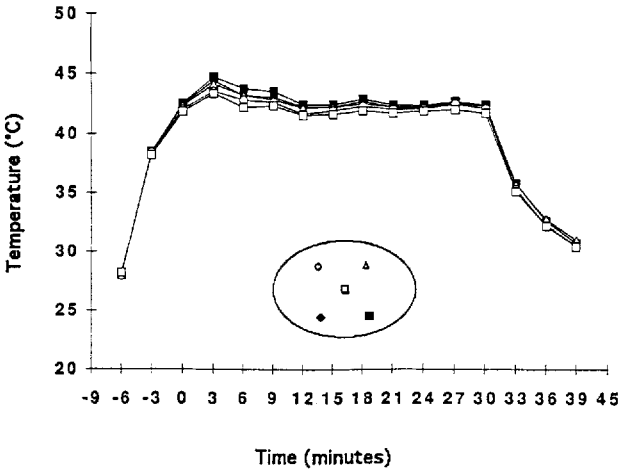

Fig. 2. Temperature/time profile for 400-mm^3 RIF-1 tumors (N = 10) heated interstitially for 30 minutes at 42°C with two 1.0-cm cylindrical diffusors. The location of the five microthermocouples is shown.

Treatment at 46°C yielded more favorable results compared to 42 and 44°C treatments. Tumors treated at 46°C for 60 minutes showed TGDs of 20.8 to 22.5 days, with a mean TGD of 21.6 ± 2.1 days. Tumors treated at 42°C for 30 or 60 minutes showed TGDs of 6.9 to 8.9 days, with a mean TGD of 7.7 ± 0.75 days; 44°C treatments for 30 or 60 minutes resulted in TGDs of 8.3 to 11.4 days, with a mean TGD of 9.7 ± 1.3 days. Tumor response

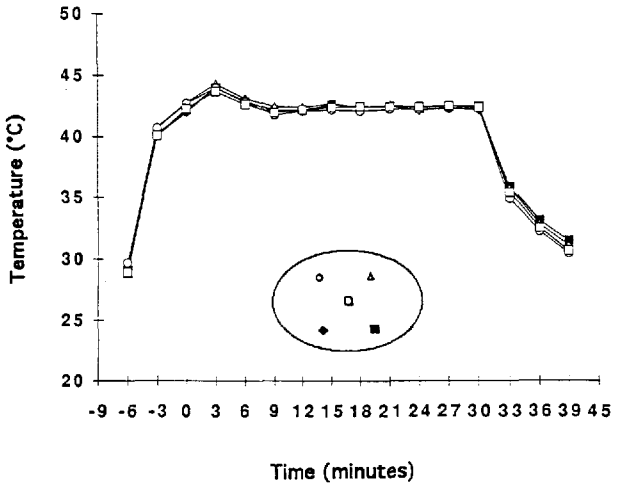

Fig. 3. Temperature/time profile for 400-mm^3 RIF-1 tumors (N = 10) heated interstitially for 30 minutes at 42°C with three 1.0-cm cylindrical diffusors. The location of the five microthermocouples is shown.

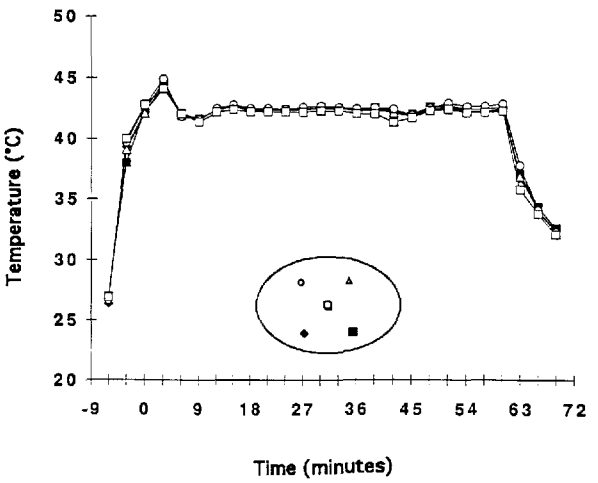

Fig. 5. Temperature/time profile for 400-mm^3 RIF-1 tumors (N = 10) heated interstitially for 60 minutes at 42°C with four (4) 1.0-cm cylindrical diffusors. The location of the five (5) microthermocouples is shown.

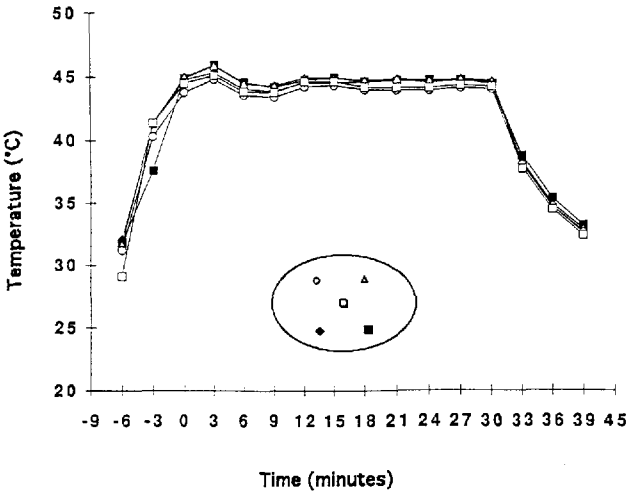

Fig. 4. Temperature/time profile for 400-mm^3 RIF-1 tumors (N = 10) heated interstitially for 30 minutes at 44°C with four 1.0-cm cylindrical diffusors. The location of the five microthermocouples is shown.

was not only affected by tumor temperature, but by the number of implanted fibers; four fiber treatments at each temperature resulted in the greatest TGD compared to two or three fiber treatments. Various changes in laser Doppler flow were also seen in response to heat treatment (Table 2). A significant change (decrease) in LDF was observed after 42°C treatment (versus before heating) using two fibers for 30 minutes ($P < .05$), and following 44°C treatment (compared to LDF before heating) using four fibers for 60 minutes ($P < .05$). Significant changes ($P < .05$) were also seen for both groups treated at 46°C; LDF was measured on the surface of the skin and in the center of the tumor for these groups.

Tumors treated at 46°C showed the most favorable TGD as well as a greater skin necrosis 48 hours post-treatment (73%). Although resulting in a more favorable TGD than those treated at 42°C, tumors treated at 44°C presented with skin necrosis 48 hours post-treatment identical with tumors treated at 42°C (approximately 56%). Following dissection, it was determined that the areas of necrosis in the tumor were centered around the areas (tracks) along each fiber location. Little gross tissue necrosis was seen in those areas more than 2.0 mm away from each fiber location.

DISCUSSION

The most important objective of this study was to determine if hyperthermia induced interstitially by a Nd:YAG laser coupled to multiple fiberoptics was effective in the treatment of RIF-1 tumors. Owing to a unique microprocessor-controlled temperature feedback system, treatment temperature was maintained at desired levels over the course of heat treatment (30 or 60 minutes). Previous experiments [4,6] have shown that interstitial treatments using a single fiber at temperatures ranging from 42 to 50°C resulted in tumor growth delay but no extensive control of the RIF-1 tumor. This study focused on multiple fiber

TABLE 2. Mean Laser Doppler Flow (LDF) in RIF-1 Tumors Before and After Nd:YAG Laser-Induced Heating

Temp./Time/No. fibers	N[a]	Mean LDF ± SD before heat[b]	Mean LDF ± SD after heat[c]
42°C/30′/2	10	2.08 ± 0.41	1.61 ± 0.61[d]
42°C/30′/3	10	2.57 ± 0.90	2.75 ± 1.52
42°C/30′/4	10	1.92 ± 0.64	1.87 ± 0.53
42°C/60′/4	10	2.15 ± 0.69	2.03 ± 0.83
44°C/30′/2	10	2.26 ± 0.62	1.96 ± 1.07
44°C/30′/3	10	2.01 ± 0.81	1.72 ± 0.88
44°C/30′/4	5	2.17 ± 0.54	4.20 ± 1.26
44°C/60′/4	10	2.15 ± 0.76	1.63 ± 0.91*
46°C/60′/4	15	2.09 ± 0.40	0.91 ± 0.24[d]
46°C/60′/4	15	2.58 ± 1.02[e]	1.78 ± 0.90[e]

[a]No. of RIF-1 tumors treated using multiple-fiber heating.
[b]Mean LD flow (±SD) measurement taken on the skin surface overlying the tumor in 3-minute intervals for 30 minutes prior to treatment.
[c]Mean LD flow (±SD) measurement taken on the skin surface overlying the tumor in three minute intervals for 30 minutes post-treatment.
[d]Mean LD flow (±SD) measurement taken on the skin surface overlying the tumor in 3-minute intervals for 60 minutes post-treatment.
[e]Mean LD flow (±SD) measurement taken in the center of the tumor in 3-minute intervals for 30 minutes prior to (before heat) or 60 minutes post-treatment (after heat).
*Differences between mean LDF before and after heat treatment of these groups was significant ($P < .05$) using a two-tailed t-test; results from other groups were not significant.

treatments at 42, 44, and 46°C to determine what, if any, effect a greater number of fibers and increases in tumor temperature would have on RIF-1 tumor response.

The Nd:YAG has the ability to penetrate tissues due to a relatively weak absorption by major tissue components [6]. Previous studies have shown the efficacy of hyperthermia in the destruction of malignant cells and the disruption of pathways essential to cellular survival [3,8,10]. Through the use of a Nd:YAG laser working in conjunction with a prototype beam splitter device, it was possible to use multiple fibers (2, 3, or 4) as opposed to a single fiber for the induction of hyperthermia. This system also utilizes a microprocessor-controlled temperature feedback system, which monitors temperature output from each fiber and automatically adjusts laser output, increasing or decreasing it, to prevent any significant deviation from the desired, pre-set temperature. As seen in Figures 2–5 and in Table 1, this system is capable of providing a constant temperature during heat treatment. As seen in Table 1, the mean treatment temperatures were consistent within 0.1–0.2°C of each other. In the temperature/time profiles (Figs. 2–5), which show the mean temperature at each fiber, it was observed that the desired temperatures were achieved for each fiber, and these temperatures were consistent at all thermocouple locations throughout the tumor. No tissue area near any fiber reached a temperature that was considerably higher or lower than at any other fiber location. Before the desired temperature was reached, though, each fiber location briefly reached a temperature that was approximately 2.0 to 3.0°C higher than the desired temperature before leveling off.

Heat treatments described here were performed at 42, 44, or 46°C, with two, three, or four fibers inserted into the tumor. Heat was delivered for 30 minutes (two, three, or four fibers) or 60 minutes (42 and 44°C, all 46°C treatments used four fibers only). Although no tumor control at 60 days was observed, the use of multiple fibers for the induction of hyperthermia resulted in a marked improvement in tumor growth delay compared to previous results using a single interstitial fiber [6,16]. Treatments at 42 or 44°C resulted in improved growth delays compared to previous single-fiber treatments, with growth delays improving both with temperature and the number of fibers used to deliver heat (Table 1). Statistically insignificant correlations were observed between temperature, the number of fibers used, and the mean tumor growth delay (data not shown). Weak correlations were also seen between the amount of necrosis present on the tumor surface and the mean tumor growth delay (data not shown). In comparison to lower temperatures, treatments at 46°C for 60 minutes resulted in growth delays improved by 50–70%, with weak correlations seen between temperature, power, the amount of necrosis present, and mean tumor growth delay (data not shown). These correlations, though not of statistical significance, suggest that optimal treatment would result from higher temperatures, using four fibers.

No correlation was seen between tumor growth delay and total dose (J/cm) delivered to the tumor, which has been observed in previous studies [5,6,16]. When attempting to increase the temperature of the tumor to one that will cause cytotoxicity, it is the actual temperature achieved which is critical, not the total number of photons (dose) delivered.

Skin laser Doppler flow (LDF) was also measured before and after heating in order to deter-

mine regional vasculature effects of hyperthermia (Table 2). LDF measurements were taken on the overlying skin of the tumor in 3-minute intervals for 30 minutes prior to treatment and every 3 minutes for 30 minutes following treatment. For those treatments at 46°C, LDF measurements were taken on the overlying skin and in the center of the tumor every 3 minutes for 60 minutes following treatment. The decision to measure skin LDF was not made until after treatment had begun for the first group (those treated at 44°C with four fibers for 30 minutes). This accounts for the discrepancy in the number of tumors measured in this group compared to the other groups. Results from these experiments showed significant changes ($P < .05$) in skin LDF pre- and post-heating for four treatment groups: Those treated for 30 minutes with two fibers at 42°C, those treated for 60 minutes with four fibers at 44°C, and both groups treated at 46°C. The other treatment groups showed weaker correlations between changes in laser Doppler flow and tumor growth delay. Regardless, tumor control was not achieved in those treatment groups showing significant decreases in LDF post-heating. This suggests that the location of the LDF probe on the skin surface does not accurately represent changes in laser Doppler flow within the tumor after heat treatment. The laser Doppler probe was originally placed on the overlying skin to prevent further trauma (in addition to fibers and thermocouples) to the tumor during treatment. Although the probe was placed in the center of the tumor for 46°C treatments, with readings taken for 60 minutes, tumor control was not observed despite the significant decrease in laser Doppler flow in comparison to those readings taken on the surface of the skin. It would appear from this data, then, that the LDF probe must be placed in the center of the tumor and measurements must be taken over a longer period of time following treatment to record a more prognostic change in laser Doppler flow.

In conclusion, we have shown that hyperthermia induced by a microprocessor-controlled Nd:YAG laser using multiple fibers produced improved results compared to previous single-fiber treatments [4,16]. Tumor response was dependent on the number of fibers and the treatment temperature, with the most favorable results seen following treatment at the highest temperature (46°C) using the maximum number of fibers (4). The results of these experiments support several other studies in which hyperthermia was shown to slow or control tumor growth [3,4,8,17]. Delivering near-infrared radiation using multiple fibers may be useful for controlled uniform heating of certain hard-to-treat human tumors (e.g., liver, esophagus, eyes). This method of treatment allows for precise temperature control and delivery of heat, thus sparing surrounding healthy tissue from unnecessary damage. Our long-term goal is to determine the treatment parameters that will result in significant long-term control in our heat-resistant tumor model. Changes (increases) in treatment duration should improve our tumor response results. Laser Doppler flow measurements post-heating are now being recorded within each tumor and for a longer period of time post-heating (90 minutes); it is hoped that this may provide a more accurate indication of initial vasculature damage in the tumor, which may be more predictive of long-term tumor response.

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